VI. EFFECT OF INTRAVENOUS INJECTIONS OF AGAR

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SYNOPSIS

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SUMMARY

In view of the fact that agar, when added to a serum, readily produces anaphylatoxin, it seemed rational to expect that a like result could be obtained in vivo. In other words, it should be possible to produce a typical anaphylatoxic poisoning, that is, the anaphylactic shock, by an intravenous injection of an agar solution or suspension. The early tests made with this object in view were far from encouraging, for the reason that they were made at a time when the optimal conditions for the in-vitro production of agar anaphylatoxin were still unknown. In the end the desired result was attained; not only was it possible to secure perfect anaphylactic shock in guinea-pigs, but also corresponding reactions in rabbits and rats. Furthermore, and as an offset to the possible objection that such shocks were due to mere agar embolism, it was shown by transfusion experiments that a typical anaphylatoxin was formed in the blood of the injected rats and guinea-pigs, and possibly in that of similarly treated rabbits.

INJECTION OF GUINEA-PIGS

Agar Sol.—In one series of experiments the agar was injected as a sol at a temperature of 50 C. This temperature was employed with the idea in mind that the more perfect the state of dispersion, the

better would be the result. Previous in-vitro tests had shown that the 50 C. sol was capable of producing naphylatoxin. For these tests, the sterile 0.5% agar was liquefied by heating in the water bath at 100 C. for about 15 minutes, after which it was placed in a Roux bath at 50 C. for a like time. The hydrosol was then injected, intravenously, in varying amounts, as indicated in Table 59, which also gives the speed of the injection.

TABLE 59

INJECTION OF 0.5% AGAR HYDROSOL (50 C.) INTO GUINEA-PIGS

Guinea-Pig		Agar Hydrosol		Result
No.	Weight	c.c. (intravenously)	Injection Time (sec.)	
1	210	1.0	20	Nil
2	215	1.5	28	Few jerky spasms
3	183	2.0	25	Increased respiration
4	207	3.0	35	Increased respiration and few slight jerks
5	210	5.0	40	Nil
6	240	7.5	45	Increased respiration, slight peripheral irritation
7	215	10.0*	65	6'20". Typical shock and autopsy

* This represents 232 mg. of dry agar per kilo of body-weight.

It will be seen from the table that of 7 guinea-pigs which received varying amounts of sol ranging from 1 to 10 c.c. only the one receiving the latter amount died. In this animal (No. 7) the shock was perfectly typical, with dyspnea, spasms, and convulsions; the autopsy was likewise, showing maximal distention of lungs, heart beating, blood fluid, and no clot.

Since the death of Guinea-pig 7 was so clearly one of typical anaphylactic shock, confirmatory results were confidently expected. But a new agar solution, prepared and tested on the following day, gave most disappointing results. Of 6 guinea-pigs which received an intravenous injection of 10 c.c. of the 50 C. sol, 5 showed no other effect than slightly increased respiration; one died in 1 minute 50 seconds, in an atypical manner supposedly due to the rapid injection (35 seconds) of the hot sol. The single typical result was therefore rather exceptional and was probably dependent on some slight condition easily overlooked.

It is worth noting that frequently 10 c.c. of agar sol, representing 50 mg. of agar (200-250 mg. per kilo), can be given without any ill effect. It will be shown that with the agar in the proper state even as little as 9.4 mg. per kilo of body weight may be fatal.

The injection of hydrosol at 37 C. (obtained by placing the liquefied agar in the Roux bath for several hours) likewise gave little or no

result. At most a few slight jerks would follow the injection of from 5 to 10 c.c. of such sol.

Agar Gel.—Other attempts were made to produce shock by injection of suspensions of solid gel in 0.85% salt solution. These were made when experience had shown that the gel was more reliable than the sol as a producer of anaphylatoxin. The previously liquefied agar was first placed in cracked ice to gel, and the solid mass on shaking readily changed into a semifluid state. When 1 part of this semigel was shaken with 4 parts of salt solution, it yielded a fine suspension which could be readily injected intravenously. Such suspension, when slowly injected in 2-c.c. amount into a 230-gm. guinea-pig, produced marked peripheral irritation, defecation, and slight jerky spasms, besides a drop in temperature to 36 C. Three cubic centimeters of the same suspension produced death in a 230-gm. guinea-pig in 1 hour; while a like amount in another animal of 190 gm. caused death in 2 minutes. The symptoms and findings were not those of typical anaphylaxis (lungs collapsed, clot in heart).

On another occasion tests were made with a like suspension which had been vigorously shaken for 3 minutes and then iced for 15 minutes. As much as 7.5 c.c. of this were injected into a 270-gm. guinea-pig without producing death, tho the shock was severe and recovery slow. The same dose of another suspension, made on the same day, killed in 3 minutes, but here, as before, collapse of lungs and heart clots showed that the object was not attained. Injections of 3 and 5 c.c. of this suspension resulted at most in a few slight jerky spasms and a slight dyspnea.

Agar Sol-Gel.—It was evident from the inconstant results aforementioned, that a perfectly homogeneous suspension of the gel could not be obtained by mere shaking of the semifluid mass with salt solution, and that as long as gross particles of agar were present, an atypical shock would be produced. Accordingly, what seemed to be a more promising method was tried.

The agar after being liquefied by heating at 100 C. for 15 minutes was placed in a Roux bath at 37 C. for 2 hours to form a hydrosol. This was then added to one or more parts of salt solution, also at 37 C. and thoroughly shaken for 5 minutes, after which the resulting fluid was placed in cracked ice for 1 hour. It remained perfectly clear with no sign of agar clumps. In this liquid the agar is therefore in an extreme state of dispersion or division and what is very important, these particles are presumably in the gel form. The results (Table 60) obtained with this sol-gel met all expectations.

TABLE 60

INJECTION OF AGAR SOL-GEL (0 C.) INTO GUINEA-PIGS
(THE SOL AND SALT SOLUTION, BOTH AT 37 C., WERE MIXED AND ICED FOR 1 HOUR)

Mixture	Guinea-Pig		Agar Sol-Gel			Result
	No.	Weight	c.c. (intravenously)	Agar* (mg.)	Injection Time (sec.)	
1:1	1	273	1.5	3.75	20	Slight. In ¾ minute slight jerky spasms, some dyspnea
	2	250	3.0	7.5	40	Very slight. In 6 minutes mild peripheral irritation
	3	300	5.0	12.5	60	Slight. In 1 minute few slight jerky spasms, respiration becoming rapid and shallow and later very labored
	4	325	5.0	12.5	30	7′. Atypical shock; at once on side, limp, very shallow respiration. Autopsy showed lungs partially distended, heart not beating, clot present
1:4	5	320	4.0	4.0	60	4′30″. Typical shock; no effect for 2 minutes, then peripheral irritation, dyspnea, spasms, thrown. Autopsy, 5 minutes after death, showed maximal distention of lungs, hemorrhages; heart stopped, blood fluid, no clot
	6	318	4.0	4.0	50	Severe shock. No effect for 2 minutes, then peripheral irritation, severe dyspnea, thrown in 6 minutes, but up 4 minutes later. Recovered
	7	315	5.0	5.0	50	6′. Typical shock; no effect for 2 minutes, then peripheral irritation, moderate dyspnea, spasms, thrown. Autopsy 5 minutes after death; same findings as in No. 5
	8	325	5.0	5.0	30	5′. Typical shock; peripheral irritation in ½ minute, then dyspnea, spasms, convulsions, thrown. Autopsy 10 minutes after death showed same findings as in No. 5. Very slight apex beat
	9	315	10.0	10.0	60	Very slight. Mild peripheral irritation, in 3 minutes slight dyspnea, no spasms
1:9	10	275	5.0	2.5	30	Slight. Mild peripheral irritation, few jerky spasms, labored respiration
	11	275	10.0	5.0	60	Severe. Very excited, peripheral irritation, marked dyspnea, spasms, jumps, not thrown, in 5 minutes quiet but depressed

* In the fatal cases, Nos. 4, 5, 7, and 8, the amount of agar per kilo of body weight was 38, 12.5, 16, and 15 mg., respectively.

It will be recalled from the previous work that the in-vitro sol-gel mixtures yield the most rapid production of anaphylatoxin; in this case essentially the same conditions were supplied in vivo. That a certain state of the agar, rather than quantity, is necessary to the production of a severe shock will be seen on comparison of Tables 59 and 60. Thus, 10 c.c. of the undiluted sol, at 37 C. or at 50 C., containing 50 mg. of agar, repeatedly produced little or no effect, tho in one instance (Table 59, No. 7) it did cause typical shock and death.

By contrast, in the fatal tests recorded in Table 60 (Nos. 5, 7, and 8) the amount of agar was but 4 or 5 mg. On reference to No. 8 of Table 62 it will be seen that even 2.5 mg. of agar may cause a fatal shock. Undoubtedly, the results are influenced not only by the amount and state of the agar, and the rate of injection, but also by the varying resistance of the guinea-pigs.

A striking feature in connection with the tests detailed in Table 60, and elsewhere, was a period of incubation or quiet following the injection. Sometimes no effect was noticeable for fully 2 minutes, and then the characteristic symptoms of anaphylactic shock developed. Also, it is to be noted that the speed of injection is an important factor in this work just as it is in all other attempts at producing shock, regardless of the agent employed. The rapid injection of the high concentration is responsible for the sudden and atypical symptoms and findings in No. 4, Table 60. With this one exception the symptoms and findings in the other test animals were those of typical anaphylactic shock.

TABLE 61

INJECTIONS OF AGAR SOL-GEL (0 C.) INTO GUINEA-PIGS

Exper.	Guinea-Pig		Agar Sol-Gel		Result
	No.	Weight	c.c.* (intravenously)	Injection Time (sec.)	
A	1	209	4	30?	Severe. Nil for 3 minutes
	2	220	5	"	Near-kill. Nil for 2 minutes 30 seconds
	3	206	"	15?	7′35″. Atypical shock, blood fluid, no clot
	4	235	6	?	Very slight. Nil for 3 minutes
	5	210	5	60	Very slight
	6	180	"	30	Very severe. Nil for 2 minutes
	7	182	"	15	2′. Atypical shock, clot doubtful
B	8	212	5	30	Moderate. Nil for 2 minutes
	9	203	"	"	5′20″. Nil for 2 minutes; typical shock and autopsy
C	10	207	"	"	Very slight. Nil for 3 minutes
	11	297	"	"	1′40″. Atypical shock
	12	320	"	"	Very slight. Nil for 4 minutes
	13	320	"	"	Nil
D	14	302	"	60	Slight. Nil for 3 minutes
	15	277	7.5	75	Slight. Nil for 2 minutes
	16	265	5	60	Nil
	17	250	"	"	2′15″. Atypical shock
	18	252	"	"	Moderate. Nil for 2 minutes

* The number of milligrams of dry agar injected corresponds to the number of cubic centimeters in the fatal cases, Nos. 3, 7, 9, 11, and 17; per kilo of body-weight, this corresponds to 24, 27, 24, 17, and 20 mg.

In Table 61 is recorded a series of results with sol-gel mixtures, the diluent being distilled water.

The method of preparation was exactly the same as before. The mixture consisted of 1 part of sol and 4 parts of distilled water; it was shaken for 1 minute in Expers. A and B, and for 5 minutes in Expers. C and D. After icing for 1 hour, the perfectly clear liquid was injected directly, without any shaking, in Expers. A and D; and with vigorous shaking for 1 minute in Exper. C.

Exper. A was intended to show the effect of the rate of the injection on the development of the shock. Two different mixtures, prepared in exactly the same way, were employed; one was used for Tests 1 to 3, and the other for Tests 4 to 7. It will be seen that very slow injections, requiring 1 minute or more, have little or no effect.

The controls given in Part IX may serve for a like purpose at this place; they show that the injection of 5 c.c. of distilled water per 100 gm. of body-weight is without effect; the injection of 7.5 c.c. per 100 gm., when given very slowly, is likewise without effect, but if given rapidly, it may cause an atypical shock similar to those of Nos. 3, 7, and 11, Table 61.

In Exper. B, the first test (No. 8) was made without shaking the mixture, whereas for Test 9 the same mixture, after standing in the room for 1 hour, was thoroughly shaken before injection. The fatal shock in this case is probably due to individual variation in guinea-pigs rather than to the treatment of the mixture. A perfectly typical anaphylactic shock was obtained in this test.

In Exper. C, Test 10 was made at once after the usual icing for 1 hour, the mixture being thoroughly shaken. It was then returned to 0 C. for 3 hours and when retested on No. 11 it rapidly killed. On being tested again, 17 minutes later, it had little or no effect. These results again point to the individual resistance of the guinea-pigs.

In Exper. D, the first two tests were made after icing for 1 hour; the third (No. 16) was tried after icing for 2 hours more. The mixture was then placed at 37 C. for 24 minutes and when tested on No. 17 it gave a fatal result. Retested 6 minutes later (No. 18) it again failed to give more than a moderate effect. These variations, it will be seen, cannot be accounted for except on the basis of individual susceptibility on the part of the test animals.

The atypical shock and findings observed in 3 of these animals must not be interpreted as due to an entirely different mechanism (that is, agar embolism) from that of the characteristic anaphylactic shock. Rather, it must be looked upon as a reaction of the same type but moving at an increased speed and with greater violence. Anaphylatoxin-production and fibrin-coagulation are essentially twin reactions, in which presumably a certain labile protein constituent of the plasma (or serum) of the type of fibrinogen is involved. Instant paralysis of respiration means an absence of symptoms such as peripheral irritation, spasms, and convulsions; it likewise means incomplete distention of the lungs. In extremely rapid death, a clot may be found in the heart, while in slow death it is doubtful or absent. It is noteworthy that the blood on removal to a test tube may remain perfectly noncoagulable, as was the case in No. 3, in which it remained fluid for 24 hours; in others, the blood may show a slight viscosity,

which soon passes away and leaves it perfectly fluid, or else goes on to form a minute clot, the remainder of the blood being unchanged.

The typical shock, it is seen, is ushered in with a latent period of from 2 to 3 minutes; then follows the usual train of anaphylactic symptoms—peripheral irritation, dyspnea, spasms, convulsions. These were particularly noted in Nos. 1, 2, 6, and 9, Table 61. Death in such cases always occurs in from 5 to 7 minutes, this being the sum of the latent and symptomatic periods.

The latent period has an important bearing on the mechanism of the intoxication. When an anaphylatoxic serum is injected, the latent period is short, rarely exceeding 30 seconds, which means that the ready-made poison acts immediately. When, however, the sol-gel mixture is injected, the poison must be made within the animal, and this takes an appreciable length of time. It can be shown by in-vitro experiment that agar can and does produce, at 37 C., a fatal dose of poison in less than 2 minutes. With the large amount of blood in the animal, it is obvious that several lethal doses may be produced in the same time and even in less. As bearing on this point reference is made to Table 65. It may be added that a latent period is seen in all similar intoxications, even in poisoning with minimal doses of potassium cyanid.

In the typical agar intoxications, therefore, the effects cannot be ascribed to the agar itself, but to the anaphylatoxin which is made within the blood of the animal as a result of the disturbance set up by the presence of an alien substance. In other words, the agar initiates or intensifies the reaction proper which leads to poison-production. It exerts a force comparable to the pull of a trigger; the blood vessel is the tube carrying the explosive charge. The reaction which takes place will be shown to be essentially identical with that which occurs in a sensitized animal when shocked (true anaphylaxis); and further, it will be found to be identical with that which occurs in vitro when anaphylatoxin is made in normal serum.

The relatively meager results obtained with distilled water as a diluent (Table 61) led to further work with salt solution in the hope of securing more positive effects and a better understanding of the reaction involved. It will be seen from Tables 62 and 63 that these attempts proved most satisfactory.

The experiments recorded in Table 62 were made with a sol-gel mixture in which salt solution was used in place of distilled water.

They are designated as B, C, and D and were made on the same day as the experiments with corresponding designation in Table 61. The same sol-gel was used and the conditions were the same, the experiments being made in parallel. The two tables are therefore directly comparable except in Exper. D, where the mixture used for No. 7 was iced for 2 hours longer than in the corresponding test (No. 16); Nos. 8 to 10 are not duplicated in the preceding table.

TABLE 62

INJECTION OF AGAR SOL-GEL (0 C.) INTO GUINEA-PIGS
(DILUTIONS MADE WITH SALT SOLUTION 1:4)

Exper.	Guinea-Pig		Agar Sol-Gel		Result
	No.	Weight	c.c. (intravenously)	Injection Time (sec.)	
B	1	209	5	30	Severe. Nil for 3 minutes
	2	206	"	"	5′. Nil for 2 minutes. Typical shock and autopsy
C	3	215	"	"	Moderate. Nil for 1½ minutes
	4	290	"	35	Very slight. Nil for 2 minutes 45 seconds
	5	275	"	30	6′10″. Nil for 2½ minutes. Typical shock and autopsy
	5a	310	"	"	Nil
D	6	280	"	60	Death after 2 hours. Nil for 1 minute
	7	260	"	"	Slight. Nil for 2 minutes
	8	265	5*	"	12′. Nil for 2 minutes, then typical shock and autopsy
	9	265	5*	"	Very severe. Nil for 2 minutes 30 seconds
	10	260	2.5	30	Nil

* For Tests 8 and 9 the sol-gel mixture was diluted with an equal volume of distilled water.
In the fatal cases, Nos. 2, 5, 6, and 8, the amount of dry agar, per kilo of body weight, was 24, 18, 18, and 9.4 mg., respectively.

A comparison of Tables 61 and 62 shows that the mixtures with salt solution apparently do not give an atypical shock such as was obtained with those in which distilled water was used. The symptoms and findings are those of typical anaphylactic shock. Particularly noteworthy are the last three tests. For Tests 8 and 9 the sol-gel salt mixture was diluted with an equal volume of distilled water; 5 c.c. of this, tested at once, proved fatal in No. 8, and the test repeated 45 minutes later gave a very severe shock (No. 9), while the undiluted mixture was without effect in No. 10. The dilution of the mixture seemed to have a beneficial effect, and it may be added that similar favoring action has been observed with other agents.

With reference to these tests, it is important to note that even as small an amount as 2.5 mg. of agar is capable of producing a fatal anaphylactic shock (No. 8). Doerr and Russ[1] found colloidal silicic acid to kill in dose of from 5.6 to 7 mg. While Friedberger and Tsuneoka[2] were unable to produce death with kaolin in dose less than 15 mg. Consequently, it is seen that apparently so harmless a substance as agar, in the proper state, can be more toxic than either of these colloids.

The toxicity of kaolin-treated serum was at one time ascribed by Friedberger,[3] not to the formation of anaphylatoxin, but to the suspended kaolin, and he explained the effect on the temperature after injection as due to injury of the endothelial lining of the blood vessels by the sharp corners of the kaolin, and death he considered as due to embolism. The fact that inactivated treated serum with kaolin had no effect was explained with equal facility by assuming that the sharp corners had become coated with coagulated albumin. Eventually, with Tsuneoka he realized that the kaolin toxicity was not mechanical, and to account for the effects he felt obliged to imagine that they depended on the absorption of certain constituents of vital cells!

The fact that the 10% sol-gel mixture (D, No. 8) killed in a dose containing but 2.5 mg. of agar, suggested a comparative trial of 10, 15, and 20% mixtures. Two such series of trials were made and the results are given in Table 63. The mixtures were prepared by adding 6 c.c. of the 37 C. sol to 54, 33.6, and 24 c.c. of salt solution, respectively, the diluent having been previously warmed to 37 C. After thorough shaking for 5 minutes, the mixtures were placed in cracked ice for 1 hour; the tests A and B were then made.

The smallest amount of agar which proved fatal in these experiments was 3.75 mg. (Nos. 2, 3, 9, and 14). Possibly better results are obtainable with fresh mixtures iced for only half an hour. On the whole, the results were much better than those in previous trials; of the deaths, only one was atypical.

It would be difficult to say which dilution gave the best results. It was believed at the time that the variations seen, for example, in Exper. B, Nos. 9, 10, and 11, were due to a loss in toxifying power

[1] Wien. med. Wchnschr., 1912, 25, p. 338.

[2] Ztschr. f. Immunitätsf., 1913, 20, p. 405.

[3] Centralbl. f. Bakteriol., R., 1913, 57 (Beiheft) , p. 242; that is, Bericht üb. d. 7t. Tagung d. Fr. Ver. f. Mikrobiol. in Berlin, 1913. Ibid., 1912, 54 (Beiheft- 6t. Tagung), p. 251.

because of the time which had elapsed between the tests; these tests were made 18 and 11 minutes apart. A similar condition was seen in Exper. C., in which the same sol-gel mixture was tested after icing for 36 and 76 minutes. It is more likely in view of results such as were presented in Table 45, that these variations were due to the inconstant behavior of the animals rather than to the slight changes mentioned.

The rate of injection, however, seems to be a factor of importance. Thus, in Test 4, the guinea-pig was given 2 injections of 5 c.c. each, 2 minutes apart. The very slight effect produced in this case is to be compared with the results of the tests immediately preceding and following (Nos. 3 and 5); the former, which was made 28 minutes before, killed, and the latter, made 11 minutes after No. 4, gave a severe shock. It would seem from this as if the divided dose exerted a distinctly minimal action. The result is in line with previous observations that large amounts slowly injected may be less dangerous than smaller quantities rapidly injected. Similar results are known to occur when peptone is injected in divided doses.

TABLE 63

INJECTION OF AGAR SOL-GEL (0 C.) INTO GUINEA-PIGS (DILUTIONS OF 10, 15, AND 20% IN SALT SOLUTION)

Exper.	Mixture	Guinea-Pig		c.c. (intravenously)*	Result
		No	Weight		
A	1:4	1	250	5	8′50″. Nil for 2 minutes, typical shock and autopsy
	1:5.6	2	265	"	9′30″. Nil for 2 minutes, typical shock and autopsy
		3	255	"	8′45″. Nil for 4 minutes, typical shock and autopsy
		4	265	10	Very slight
		5	250	5	Severe. Nil for 3 minutes
	1:9	6	248	"	Slight. Nil for 4 minutes
		7	256	10	4′40″. Nil for 2 minutes
B	1:4	8	285	5	6′25″. Nil for 1½ minutes, typical shock and autopsy
	1:5.6	9	297	"	13′. Atypical shock, blood fluid, no clot
		10	315	"	Severe. Nil for 1½ minutes
		11	310	"	Slight. Nil for 2½ minutes
	1:9	12	286	10	5′10″. Nil for 1½ minutes, typical shock and autopsy
		13	245	5	Slight. Nil for 3 minutes
C	1:5.6	14	320	"	5′30″. Nil for 3 minutes, typical shock
		15	320	"	Moderate

* The injection time for the tests was 1 minute for 5 c.c. and 2 minutes for 10 c.c.
The amount of dry agar, per kilo of body weight, in the fatal cases ranged from 11 to 20 mg.

INJECTION OF RABBITS

Only 2 tests were made to ascertain the effect produced by agar when injected intravenously into rabbits. A more extended effort would have given in all probability as positive results as in the case of the guinea-pigs. However, the findings were sufficient to indicate that agar does exert a similar action.

The sol-gel mixtures for these experiments were made by adding 1 part of sol to 9 parts of salt solution, both previously having been kept at 37 C. After vigorous mixing for 5 minutes, the sol-gel was placed in cracked ice for half an hour. This dilution, as previous experiments had shown, was fatal to guinea-pigs in dose of about 40 c.c. per kilo. The same mixture as used for Exper. 2 was injected in dose of 10 c.c. into Rat 16 (Table 64). This dose, which represents 60 c.c., or 30 mg. of agar per kilo, produced but a moderate effect in the rat, whereas Rabbit 2 died after receiving approximately half this amount. It would seem therefore that the rabbit holds an intermediate place between the guinea-pig and the rat.

Exper. 1.—A rabbit weighing 1450 gm. was given 30 c.c. of the mixture (=20.5 c.c. per kilo); in 2 minutes it showed marked peripheral irritation, but otherwise nothing.

Exper. 2.—The rabbit in this test weighed but 350 gm. It received 11.6 c.c. of the mixture, which represent 33.1 c.c. or 16.6 mg. of agar per kilo. An atypical shock resulted; the animal was at once on its side, with head drawn back, but not rigid; the respiration became deep and in 3 minutes dyspnea appeared; death occurred in 9 minutes, 30 seconds. The autopsy, made 4 minutes after death, showed partial distention of lungs, vigorous heart beat, and the blood perfectly fluid. Blood drawn from the heart and placed in a test tube clotted in 11 minutes. At 31 minutes after death the heart was still beating and no clot was present. Consequently, in this, as in many similar tests, the blood transferred from the heart to a test tube, by means of a wet syringe, clotted before that which was left in the heart. This method is therefore of little value in ascertaining whether or not the blood of a shocked animal is incoagulable. A better procedure is that which will be discussed in Part VII.

INJECTION OF RATS

The behavior of rats in response to injections of agar presents an interesting paradox corresponding with that observed in sensitized rats injected with dilute antigen or with distilled water (Part IX). It will be recalled that normal rat serum on treatment with agar yields the most powerful anaphylatoxin known, its lethal dose being from 0.2 to 0.25 c.c. per 200 gm. of guinea-pig; furthermore, it has been shown in Part V that such highly toxic sera may be injected, intravenously, in

relatively large doses into rats without producing fatal results. Weight for weight, the rat seems to tolerate more than 100 times as much of the poison as does the guinea-pig; that is, a 150-gm. rat survived 75 guinea-pig lethal doses.

On the basis that 10% of the body weight is blood, a rat weighing 150 gm. would carry 15 c.c. of blood, or 9 c.c. of plasma; were this toxified to the same degree as occurs in vitro with serum, it would yield from 36 to 45 guinea-pig lethal doses, an amount which represents from one-half to two-thirds of the dose named as nonfatal for the rat. Consequently, an additional amount of poison must be produced within the body in order to cause death, and this means, if the plasma is considered as the sole source of the poison, that the real matrix is present in greater amount in the plasma than in the serum. That such actually is the case is indicated by the high precoagulation toxicity of blood as compared with that of serum made from the same blood. For example, 2 c.c. of rabbit blood (= 1.2 c.c. plasma) just before coagulation, may be fatally toxic to a guinea-pig, whereas the serum may not be poisonous in dose of 6 c.c., 5 times the amount of the plasma which did kill. Whether the very labile fibrinogen or some constituent of the blood cells is responsible for this increased toxicity of whole blood remains to be demonstrated.

Clearly, in view of the large amount of poison necessary to kill, it should be very difficult to produce anaphylactic death, either by the injection of agar into normal rats, or by the specific anaphylactic shock induced by injection of an antigen into a previously sensitized rat. This was actually found to be the case for both of these procedures. The rat is remarkably tolerant for anaphylatoxin, whether this be injected preformed, or whether it be made in vivo as a result of treatment with agar or antigen.

The results of injections of agar into rats are given in Table 64. For these experiments, the sol-gel mixtures, prepared with salt solution in the usual way, were iced for half an hour before use The injections were made into the femoral vein. It will be noted that a number of the tests apparently had no effect, the respiratory disturbance being very slight or absent; others resulted in distinct manifestations of shock. Of the 3 deaths, 1 (No. 13) was perfectly typical both as regards symptoms and autopsy findings. The amount of agar given in the fatal injections (Nos. 5, 7, and 13) was, respectively, 10, 3.8, and 5 mg.; or better expressed, it was 80, 27, and 32 mg. per kilo of body

weight. It is evident from these tests that rats react to agar much less than do guinea-pigs, even tho the dose is relatively larger.

Inasmuch as the sol-gel mixture used for Tests 1 to 3 had very little effect, it was tried out on a 258-gm. guinea-pig in dose of 5 c.c. and was found to yield but a mild shock. Accordingly, a new solution was prepared and this, when tested at once on a 310-gm. guinea-pig in like dose of 5 c.c., produced a typical anaphylactic shock and death in 5 minutes. This active mixture was then used for Tests 4 and 5. Similar controls made after Tests 9 and 10 gave only mild effects in guinea-pigs.

TABLE 64

INJECTION OF AGAR SOL-GEL GEL (0 C.) INTO WHITE RATS
(DILUTIONS OF 10, 15, AND 20% IN SALT SOLUTION)

Mixture	Rat		Agar Sol-Gel Gel		Result
	No.	Weight	c.c. (intravenously)*	Injection Time (min.)	
1:4	1	130	2	½	Very slight. Polypnea
	2	135	3	1	Peripheral irritation; irregular respiration on side
	3	110	4	1½	Irregular respiration
	4	135	5	1	Irregular respiration
	5	125	10	1½	2′. Atypical shock
1:5.6	6	135	3	1	Nil
	7	140	5	1	6′10″. Atypical shock
	8	160	"	½	Nil
	9	125	"	¾	Increased respiration
	10	110	"	1	Increased respiration
1:9	11	132	"	1	Nil
	12	127	7.5	1	Nil
	13	155	10	½	5′30″. Nil for 2 minutes 30 seconds, then typical shock and autopsy
	14	124	"	1½	Increased respiration
	15	130	"	1½	Increased respiration
	16	165	"	1½	Increased respiration with some dyspnea
	17	150	13	1¾	Increased respiration
	18	145	15	2	Practically nil

* In the fatal cases, Nos. 5, 7, and 13, the amount of dry agar per kilo of body weight was 80, 27, and 32 mg., respectively.

The mixture employed for Tests 11 to 13, was first tested on a 320-gm. guinea-pig, which it killed in dose of 10 c.c. in 10 minutes, 30 seconds; a similar control preceded Tests 14 and 15 and here, also, the mixture was shown to be active — a 320-gm. guinea-pig responding to 10 c.c. with typical shock and death in 5 minutes, 30 seconds. Lastly, it may be stated that the mixture used for No. 16 was injected 40 minutes before into Rabbit 2; the dose of the mixture, per kilo, in the case

of the rabbit was 33 c.c. (= 16.5 mg. of agar), while that for the rat was 60.6 c.c. (= 30.3 mg. of agar). It will be seen that the rat, which received nearly twice as much of the agar as the rabbit, showed but slight effects, while the latter died. It may be further pointed out that in Test 18, the rat received 15 c.c. of a similar mixture, the injection being given in 2 minutes with practically no effect; the amount of agar injected in this case corresponded to 51.7 mg. per kilo.

There can be very little doubt but that the susceptibility of the three species tested varies as regards the injection of agar. Selecting the lowest fatal doses found, one may compare them, per kilo of animal, as follows:

Guinea-pig	9.4 mg. of agar
Rabbit	16.5 mg. of agar
Rat	27.0 mg. of agar

It is seen that the toxicity for the three animals is relatively 1:2:3. A similar relative resistance will be shown to exist in the case of poisoning with Witte's peptone (Part VII), and this fact supports the view that the intoxications by agar and by peptone are of exactly the same nature. The transfusion experiments will furnish direct evidence of this identity.

TRANSFUSION OF BLOOD OF RATS SHOCKED WITH AGAR

The previous set of experiments, taken by itself, might not be considered to bear more than a resemblance to anaphylatoxic poisoning. At first sight, the symptoms (except in No. 13) suggested little or no relation to those of shock in the guinea-pig. But it must be remembered that the rat does not behave like the guinea-pig when injected with anaphylatoxin; and, it will be shown later that this difference is equally marked when specific anaphylactic shock is attempted.

It had previously been shown that the rat could tolerate an enormous dose of anaphylatoxin and, in view of the known ease with which rat serum was toxified with agar, it was reasonable to believe that the same poison was produced in vivo. When everything is considered, the previous experiments strengthen this view. However, actual proof of the formation and presence of anaphylatoxin in the blood of the treated rats was required, and this could be supplied in but one way: by transfusing, at maximal speed, some of the rat blood to a normal guinea-pig.

It was pointed out in connection with Table 64 that, it being assumed the plasma responds to agar in the same way and to the same extent as serum, it is possible for a rat of 150 gm. to develop from 36 to 45 guinea-pig lethal doses. After an injection of 5 or 10 c.c. of the agar mixture the total volume of the blood would be 20 and 25 c.c., respectively. The transfusion of 2 c.c. of such diluted blood implies a possible transfer of from 3.6 to 4.5 guinea-pig lethal doses in the case of the 20-c.c. bulk, and of from 2.9 to 3.6 guinea-pig lethal doses in the case of the 25-c.c. bulk. Or, in round numbers, the transfusion of 2 c.c. of the blood would mean a possible transfer of 3 guinea-pig lethal doses, and, allowance being made for some in-vivo destruction of the poison, there should still be left enough to give a fair indication of its presence. This view was amply confirmed by the experiments recorded in Table 65. It may be added at this point that the same method applied to the peptone shock in rats showed that actually somewhat more than 7.5 guinea-pig lethal doses were present (Part VII).

Preliminary experiments on the transfusion of the blood of normal rats (Table 66) showed that, with fair speed, 2 c.c. of the rat blood could be transfused without producing much, if any, effect in the recipient. Hence, essentially the same negative result should follow the injection of the blood of rats injected with agar, provided the transfusion was made at the earliest possible moment. After the agar had had a chance to act in vivo for 2 or 3 minutes, which represents the latent period to which attention has been called, the blood should show the presence of the poison. And, further, in view of the fact that recovery from the shock was usually rapid, a fact implying the disappearance of the poison, it was expected that transfusion after 5 or 6 minutes would show a decrease or absence of the poison.

In Table 65 the tests are arranged according to the 'total time' required for the injection of the agar and for the transfusion of the blood; that is, the actual time from the start of the injection of the rat until the end of the injection of the guinea-pig. By 'transfer time' is meant the interval from the entrance of the syringe into the heart of the rat until its withdrawal from the vein of the guinea-pig. It represents, therefore, the maximal time any portion of the blood was in the syringe. This transfer time must be kept as short as possible, otherwise in-vitro toxification may occur (see Table 66). The difference between the 'total' and the 'transfer' time represents approximately the reaction time within the rat.

It will be seen that with a reaction time of about 1 minute (Nos. 8 and 9) the dose of blood employed contained very little poison. It is possible that a dose of 4 c.c. at this stage would have proved fatal.

With the reaction time increased to 2 minutes and especially to 3 to 7 minutes, the presence of anaphylatoxin is demonstrable either by severe or by fatal shock. With this time increased to 11 minutes, the toxicity of the transfused blood is apparently reduced to that of normal blood (Nos. 14 and 15).

TABLE 65

THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN RATS INJECTED WITH AGAR (1:9)

Exper.	Rat			Weight Guinea-Pig*	Total Time	Transfer Time	Result in Guinea-Pig
	No.	Weight	Agar (c.c.)				
A	1	170	10	200	2′35″	25″	19′. Typical shock and autopsy
	2	164	"	205	3′	25″	Good shock
	3	140	"	171	5′25″	45″	Practically nil
	4	160	"	175	6′ 5″	45″	3′ 5″. Quiet shock
	5	148	"	191	6′25″	25″	4′45″. Typical shock and autopsy
	6	155	"	184	6′30″	30″	3′30″. Typical shock and autopsy
	7	160	"	177	6′50″	30″	5′50″. Typical shock and autopsy
B	8	125	5	197	1′10″	15″	Slight
	9	148	"	148	1′30″	25″	Very slight
	10	145	"	202	3′50″	23″	4′25″. Typical shock and autopsy
	11	130	"	190	4′20″	25″	3′55″. Typical shock and autopsy
	12	115	"	181	4′55″	30″	Moderate
	13	155	"	174	7′20″	30″	4′25″. Typical shock and autopsy
	14	168	"	198	11′35″	65″	Slight
	15	170	"	185	11′40″	30″	Practically nil

* In every test 2 c.c. of heart blood were transferred to the guinea-pig.

It is significant that in both series of tests, with a reaction period of 4 minutes 40 seconds, and 4 minutes 25 seconds (Nos. 3 and 12), the transfused blood should be considerably less toxic than in the periods immediately preceding and following. It would seem as if the first production of poison was followed by a distinct drop or reversion, which in turn was followed by a second rise in toxicity, which gave way in turn to an atoxic stage. Whether the first drop in toxicity was actual, or was merely an accident due to the chance resistance of the guinea-pig used, can only be determined by repeated trials. The fact that a similar drop was observed in corresponding transfusions from rats injected with peptone (Chart 11) would seem to indicate that it expresses something more than a chance resistance.

The results given in Table 65 are expressed graphically in Chart 9, in which the abscissæ represent the reaction time.

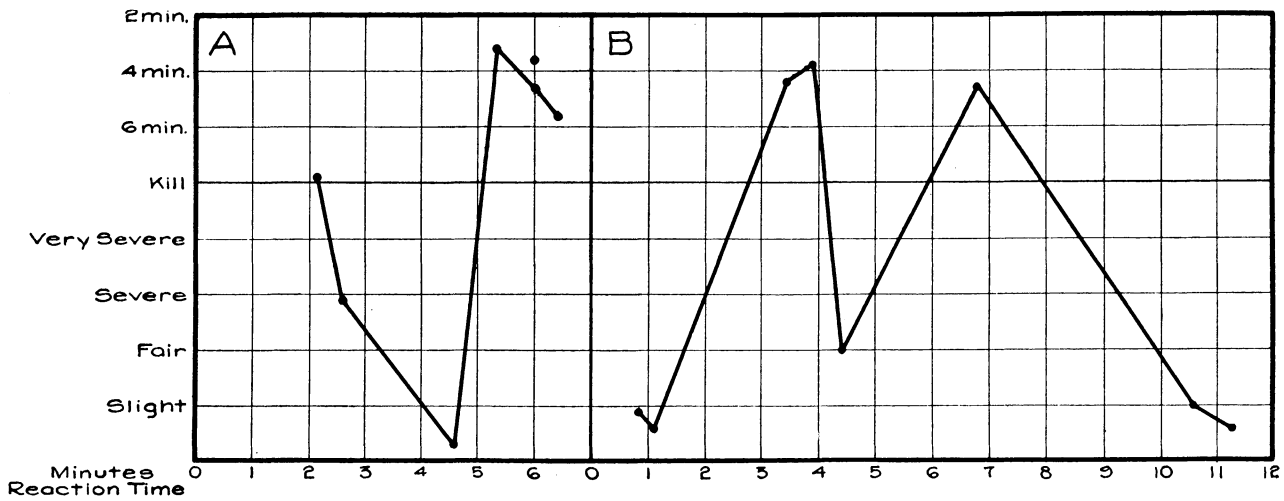

Chart 9. The in-vivo production of anaphylatoxin in rats injected with agar (Table 65).

These experiments establish beyond doubt that a poison, identical in action with the in-vitro anaphylatoxin, is produced in rats injected with agar. And, further, such poison is formed tho the rats themselves show but little effect. The autopsy findings in the guinea-pigs were those of typical anaphylactic shock with the single exception of No. 4. The fact that 2 c.c. of the blood is fatal may be taken to indicate that the injected rat contains at least 10 guinea-pig lethal doses.

It is deserving of mention that the hearts of the rats used in these tests when examined about 10 minutes after death were still beating, and the blood was perfectly fluid, with no evidence of clot. Such blood transferred to a test tube clotted in about 3 or 4 minutes.

It should be added that the sol-gel mixtures used for these tests were prepared in the usual way. One part of the sol, at 37 C., was mixed with 9 parts of the salt solution and thoroughly shaken for 5 minutes; the mixture was then placed in cracked ice for half an hour. Three such mixtures were used in Exper. A and three in Exper. B. The injections were made into the femoral vein in the rats and into the jugular vein in the guinea-pigs. The animals were immobilized, side by side, and the veins exposed before beginning the test proper. The injection time for 10 c.c. of the agar mixture varied from 1 to 2 minutes; for 5 c.c. it ranged from 15 to 40 seconds.

Transfusion of Blood of Normal Rats to Guinea-Pigs.—In Table 66 will be found the results of the transfusion of the blood of normal rats. These tests serve as controls for the experiments recorded in Table 65 and for similar transfusions. To be strictly correct, these controls should have been made with rats which had received injections of 5 or 10 c.c. of salt solution, and with reaction times corresponding to those of the experiment proper. Two such tests with egg white diluted with distilled water are recorded in Part IX. A rat was used for each experiment; after exposure of the heart, the blood was drawn up into a syringe and at once injected into the jugular of a guinea-pig.

The most important factor in these transfusions is the transfer time. It will be noted that even 4 c.c. of blood can be transfused with little or no effect provided the transfer time does not exceed 50 seconds. With a transfer time of 1 minute (No. 6), this dose of blood caused a typical anaphylactic shock. This precoagulation anaphylatoxin develops more rapidly in rat blood than it does in rabbit or guinea-pig blood (see Part VIII). This is directly due to the more rapid clotting of the rat blood.

Transfusion of Blood of Guinea-Pigs Shocked with Agar. — The successful demonstration of the in-vivo formation of anaphylatoxin in rats which had received an agar injection led to a series of similar tests with guinea-pigs. It could hardly be expected that the results would be as good in view of the fact that guinea-pig serum toxifies but feebly; and, therefore, if this was equally true for the in-vivo reaction, the guinea-pig would contain but few lethal doses. For that reason it was deemed best to transfuse as large doses of blood as possible within a transfer time not exceeding 1½ minutes. Previous tests had shown that 5 c.c. of normal guinea-pig blood, drawn from the heart and kept in the syringe for 2 minutes, produced little or no effect on subsequent injection (Part VIII). To be correct, the controls should be made with guinea-pigs injected with a like amount of salt solution.

TABLE 66

CONTROL TRANSFUSIONS OF BLOOD OF NORMAL RATS TO GUINEA-PIGS

Guinea-Pig		Rat Blood		Result
No.	Weight	c.c.	Transfer Time (min.)	
1	170	2	50	Practically nil
2	176	"	40	Very slight
3	172	"	45	Practically nil
4	205	4	45	Slight
5	210	"	48	Practically nil
6	187	"	60	Death in 3′5″. Typical shock and autopsy

For the experiment given in Table 67, the agar mixtures were prepared in exactly the same way as those used for the rat experiments. The sol-gel dilution was injected in dose of 10 c.c., and immediately thereafter the heart was exposed and 6 c.c. of blood withdrawn and injected into a new guinea-pig. Dyspnea was present in each of the 4 donors at, or before, the time of section and, according to the results in Table 63, death was expected in 5 or 6 minutes. Hence, at least 1 lethal dose of poison was in evidence, and this poison was distributed in an amount of blood corresponding to about 10% of the body-weight, plus the volume of fluid injected, that is, in about 35 to 38 c.c.

It will be seen from Table 67 that but 1 of 4 trials was successful, which may be taken to indicate that only a small amount of poison was present in the blood. The fact that 6 c.c. did kill in No. 3, means that this animal had at the time about 5½ guinea-pig lethal doses in its blood. This, it will be seen, is considerably less than the amount pres-

ent in specific anaphylactic shock induced in guinea-pigs sensitized with egg white (Part IX), in which from 14 to 24 guinea-pig lethal doses were demonstrated.

In the tests given in Table 67, the reaction time was approximately the same in all, ranging from 4 minutes to 4 minutes 25 seconds. Because of the fall in blood pressure it was undesirable to lengthen this period, unless larger animals were used as donors, and this at the time was impracticable. It is possible that, at the 4-minute period, a decrease in the amount of poison occurs, such as has been noted in rats injected with agar or with peptone (Charts 9 and 11). To decide this point it will be necessary to make additional tests with a reaction time of from 2 to 3 and 5 to 8 minutes.

TABLE 67

THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN GUINEA-PIGS INJECTED WITH AGAR (1:9)

Donor Guinea-Pig		Agar (c.c.)	Weight of Recipient*	Total Time	Transfer Time	Result in Guinea-Pig
No.	Weight					
1	270	10	199	6′	1′35″	Very slight
2	275	"	200	5′	55″	Slight
3	255	"	195	5′15″	1′15″	8′45″. Typical shock
4	280	"	175	5′35″	1′35″	Slight

* In every case 6 c.c. of heart blood were transferred to the recipient.

TRANSFUSION OF BLOOD OF RABBITS SHOCKED WITH AGAR

It has been pointed out in Part V that the rabbit, like the rat, tolerates large doses of agar anaphylatoxin, a fact which enables it to carry enormous amounts of this poison in vivo when the poison is developed as the result of injection of sheep corpuscles. It is indeed possible for a treated rabbit to have in its blood as much as 2000 guinea-pig lethal doses. Since in the rat, injections of agar result in the production of anaphylatoxin, it seemed as if the same reaction, tho perhaps less intensively, should occur in the rabbit, and if so it should be possible to demonstrate the formation of the poison by transfusion tests. It has been shown heretofore that the injection of agar in rabbits may cause typical anaphylactic shock and death, which if due to anaphylatoxin would imply the formation of considerably more than 1 guinea-pig lethal dose. The transfusion experiments seemed to be a simple matter since previous tests, some of which are given in Part VIII, showed that with attention to speed, as much as 10 c.c. of rabbit blood could be transferred without serious results.

Accordingly, transfusion experiments were made on 6 rabbits after they had received, intravenously, varying amounts of sol-gel salt mixtures.

The latter were prepared as heretofore; that is, the agar sol at 38 C. was added to 4 or 9 parts of 0.85% salt solution, and the mixture after thorough shaking for 5 minutes was placed in cracked ice for an hour or more. For each test, 2 c.c. of blood were drawn by heart-puncture, through the thoracic wall, and at once injected into the jugular vein of a guinea-pig; the transfer time—that is, from the start of the drawing of the blood until the end of the injection of the recipient—usually ranged from 12 to 20 seconds, tho occasionally it took 30 seconds. Before injecting the agar solution, as a control precaution, the toxicity of the heart blood was tested on guinea-pigs; 2 transfusions, each of 2 c.c. were made, and if the effects thus produced were but slight the injection of the agar was then carried out. The transfers then followed at intervals which are designated as 'total time'—that is, from the start of the agar injection until the end of the injection of the guinea-pig. The difference between the transfer and total times represents the reaction time during which the agar has acted on the rabbit blood. The agar injections in rabbits always caused more or less respiratory trouble but all 6 used for these tests recovered.

Exper. 1.—Two preliminary controls showed but slight effects. The rabbit (2250 gm.) was then injected with 45 c.c. of the iced sol-gel salt mixture (1:4), the injection time being 2 minutes 5 seconds. This dose represents 45 mg. of agar, or 20 mg. per kilo. The total-time transfers were made at 3′32″, 5′17″, 8′11″, and 11′21″, with but moderate results. Apparently, there was no marked increase in the toxicity of the blood.

Exper. 2.—The two preliminary tests showed moderate shock effects. The rabbit (2040 gm.) was then given 40 c.c. of a sol-gel salt mixture (1:9), the injection time being 1 minute 35 seconds. The dose given represents 9.8 mg. of agar per kilo. The total-time transfers were made at 3′ 9″, 6′ 5″, 8′ 7″, 11′ 13″, and 16′ 58″. The second of these resulted in a typical shock and death in 4 minutes, 10 seconds; the other tests were apparently of increased severity compared with the preliminary tests.

Exper. 3.—A single preliminary test gave but slight effects. The rabbit (900 gm.) then received 9 c.c. of a sol-gel salt mixture (1:4), the injection time being 1 minute. This dose corresponds to 10 mg. of agar per kilo. The total-time transfers were made at 2′ 30″, 5′ 30″, 9′ 45″, 13′, 16′, and 22′. Of these, the third resulted in typical shock and death in 2 minutes, 27 seconds, and the fourth caused a very severe shock; the others had but slight effects.

Exper. 4.—No preliminary tests were made. The rabbit (2380 gm.) was given 40 c.c. of a sol-gel salt mixture (1:4) in 2 minutes, 33 seconds. The amount of agar was 17 mg. per kilo. The transfer made at 4 minutes, 9 seconds proved fatal in 6 minutes, 29 seconds; that at 6 minutes, 42 seconds gave a very severe shock, as did also that at 14 minutes, 50 seconds. Three other transfers at 10′ 50″, 19′ 20″, and 24′ 30″ had but slight effect.

Exper. 5.—No preliminary tests were made. The rabbit (2340 gm.) received at once 23 c.c. of a sol-gel salt mixture (1:4) in 1 minute, 50 seconds. The amount of agar given was 10 mg. per kilo. The transfer at 18 minutes caused typical shock and death in 3 minutes 50 seconds, while that at 22 minutes was severe. The 5 other tests made at 3, 6, 10, 14, and 26 minutes had slight or no effects.

Exper. 6.—See Table 68. After 2 preliminary tests (Nos. 1 and 2) the rabbit of 2300 gm. was given 23 c.c. of a sol-gel salt mixture (1:4), this amount representing .10 mg. of agar per kilo. It will be seen from the results of this experiment as tabulated, that apparently the toxicity of the blood had been increased after the injection of the agar suspension since 4 of 9 tests proved acutely fatal. Typical acute shock was developed in these fatal cases.

On summing up the 6 experiments it will be found that 8 out of 37 tests, or 21.6%, were fatal, the amount of blood transferred in each case being but 2 c.c. The number of deaths could have been materially increased by the injection of a larger amount of blood, but this was inadvisable because of preliminary transfusion tests with rabbits given injections of plain salt solution. Such tests were made with the object of having rigid controls for the agar experiments proper which were to follow.

TABLE 68

IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN RABBIT INJECTED WITH AGAR (1:4) EXPER. 6

Guinea-Pig		Rabbit Heart Blood			Result in Guinea-Pig
No.	Weight	c.c.	Total Time	Transfer Time	
1	215	2		33″	Slight
2	213	"		15″	Slight
3	207	"	4′18″	13″	5′52″
4	195	"	7′22″	27″	Slight
5	191	"	12′ 8″	28″	2′47″
6	197	"	16′40″	27″	Very slight
7	205	"	22′25″	20″	4′50″
8	195	"	28′23″	18″	Slight
9	187	"	33′20″	20″	3′55″
10	187	"	38′49″	10″	Very slight
11	186	"	47′10″	15″	Fair

In the first control of this type, a rabbit of 1076 gm. received intravenously in 2 minutes, an injection of 20 c.c. of iced salt solution. The total-time transfer at 3 minutes, 55 seconds of 5 c.c. of heart blood caused typical shock and death in 4 minutes, 35 seconds, while a like amount transferred at 5 minutes, 37 seconds had but slight effect. Transfers of 3 c.c. were then made at 14 minutes and at 16 minutes, 30 seconds, which had also slight or moderate effects. Inasmuch as this experiment seemed to show that the injection of salt solution resulted in the production of some poison, similar tests were made with 3 other rabbits, the amount of blood transferred being reduced, however, to 2 c.c.

In a second control a rabbit of 1068 gm. was given 10 c.c. of a salt solution in 55 seconds, and 2 c.c. of blood transfused at 2 minutes, 37 seconds and 6 minutes, 37 seconds had but very slight effect. Similarly, in the case of a third control rabbit of 2050 gm., which received 40 c.c. of the salt solution in 1 minute, 55 seconds, total-time transfers at 3′ 45″, 6′ 45″,10′ 30″, and 13′ 20″ caused very slight or no disturbance. Likewise, transfusions from a fourth control rabbit of 2078 gm. injected with 40 c.c. of the salt solution, gave no effect at the 4′ 10″ and 9′ 10″ periods. It will be seen, therefore, that 8 transfusions, each of 2 c.c., from 3 rabbits, caused relatively little or no disturbance and this fact led to the adoption of this dose of blood in the experiments with agar.

The control transfusions after injection of salt solution would seem to justify the belief that the results obtained with like transfusions after the injection of sol-gel salt mixtures were due to anaphylatoxin produced in vivo. Strictly speaking, however, this conclusion is open to question in view of the wholly unexpected results which were obtained with 3 rabbits in tests made on the same days as the preceding. The direct transfusion of 2 c.c. of heart blood from these caused typical shock and death (Table 69). These results were the more surprising since at various times during the past year or two, transfusions of from 5 to 10 c.c. of normal rabbit blood were found to be without effect. They show clearly that the so-called normal animal may possess blood of varying toxicity. These animals seemed to be perfectly healthy, and had never been used for experimental work. The rabbits which received injections either of agar or of salt solution, were undoubtedly in a similar, tho not as marked, state of autotoxicity, and for that reason the results obtained must remain in suspense.

The cause of this unusual toxicity cannot be definitely established. It certainly cannot be ascribed to malnutrition; the possibility of an intercurrent unrecognized epidemic may be conceded, tho highly improbable because of the perfect condition of the stock animals. The fact that these experiments were made in mid-winter (January) might suggest a seasonal state of the blood, but this was offset by the records of the previous January, when the harmlessness of rabbit blood had been first established. The only essential difference would seem to be in the age and size of the animals. For the foregoing tests, young rabbits of from 1 to 2 kilos were used, whereas formerly considerably larger animals were employed.

TABLE 69

CONTROL TRANSFUSIONS OF BLOOD OF NORMAL RABBITS TO GUINEA-PIGS; SERUM TOXICITY

Rabbit		Guinea-Pig		Rabbit Heart Blood		Result in Guinea-Pig
No.	Weight	No.	Weight	c.c.	Transfer Time (min.)	
1	1335	1	206	2	22	5′
2	2275	2	200	"	15	5′40″
		2a	205	"	15	5′
3	2060	3	195	"	12	3′
		3a	202	"	12	3′10″
				Serum		
"		4	195	1.0		2′20″
		5	193	1.0		5′10″
		6	210	0.75		Slight

The results given in Table 69 are deserving of attention since they show that transfusion of even 2 c.c. of heart blood may prove fatal to the guinea-pig. The death is not due to the mere speed of the injection since it will be shown that the serum of such rabbits was actually toxic in dose of 1 c.c. (Nos. 3 and 3*a*). In other words, the blood in these animals possessed an inherent toxicity. On the assumption that the blood makes up 10% of the body weight, the animals carried, respectively, 66, 114, and 103 guinea-pig lethal doses, which corresponds for all three with 50 guinea-pig lethal doses per kilo, or with 10 such doses per 200 gm. of rabbit. It has been shown in Part V that the normal rabbit can tolerate the injection of 13.4 guinea-pig lethal doses per 200 gm., and further that the immunized cachectic rabbit may carry 240 of such lethal doses per 200 gm.

The toxicity of the heart blood in these instances cannot be ascribed to ex-vivo precoagulation changes, for these are excluded by the speed of the transfer, and especially by the fact that such heart blood defibrinated very slowly when beaten with a glass rod; the yield of fibrin was unusually small and even more striking was the observation that the relative amounts of corpuscles and serum, after centrifugation, were distinctly abnormal, the ratio being about 1 : 3. This was true whether the blood was drawn from the heart or from the carotid, as was done in the case of Rabbit 3. The blood was clearly in an altered state, which in some way is related to, and probably conditions, the toxicity observed.

It will be seen that the serum of Rabbit 3, tested within 15 minutes after the withdrawal of the blood from the heart, was toxic in dose of 1 c.c. (Nos. 3 and 3*a*). The serum of only 1 of the 3 rabbits was tested, but without doubt that from the others would have been equally toxic. Hence, here are 3 normal rabbits capable of yielding sera which are fatal in dose of 1 c.c. per 200 gm. of guinea-pig. Only one other instance of normal rabbit serum having this high degree of toxicity (0.5 per 100 gm.) has been reported. Mita and Ito[4] found 1 of 16 rabbits tested, to yield a serum of this potency.

This condition in the normal rabbit serves to establish the important fact that blood changes accompanied by increased toxicity may occur in apparently healthy normal animals; and that this reaction may become accelerated or intensified by immunizing injections. In the former some unknown cause, corresponding to the immunizing injec-

[4] Ztschr. f. Immunitätsf., 1913, 17, p. 586.

tions, serves to develop the anaphylatoxin. The natural tolerance of the rabbit toward the anaphylatoxic disturbance insures it a resistance to conditions which rapidly prove fatal to the nontolerant or susceptible guinea-pig. This explains, perhaps, why it is practically impossible to keep guinea-pigs for any length of time in cold basement rooms, tho rabbits seem to thrive there perfectly.

The acquired or inherent anaphylatoxin of the normal or treated rabbit must be identified with the precoagulation anaphylatoxin which forms in drawn blood; and also with the induced anaphylatoxin which develops in sera treated with alien substances. The three conditions express, in reality, one and the same thing — an intramolecular change in a very labile plasma constituent. When the inherent toxicity is high, there is no increase during the coagulation of such blood; when it is at a minimum, as when 10 c.c. are nonfatal, the precoagulation toxicity becomes marked. In other words, the matrix is available in the latter for transformation, while in the former it has already undergone change.

AGAR AND ENDOTOXIN

A brief mention can be made at this point of the classic work of Pfeiffer[5] on cholera immunity. He found that the intravenous injection of 0.5 mg. of live cholera bacilli per 100 gm. of guinea-pig, caused death. The dose for the organism killed by chloroform treatment for 10 minutes was 0.75 mg. The symptoms following such injections into immunized, that is sensitized, guinea-pigs were those of typical acute anaphylactic shock, while those in normal animals were similar but slower, with characteristic fall in temperature. These effects, as is well known, were ascribed to the presence of an 'endotoxin.'

Friedberger and Mita[6] found that the moist culture of Vibrio metschnikovi, killed by heating at 60 C. for 2 hours, was fatal in dose of 0.25 gm. per 100 gm. of guinea-pig. When sensitized by a previous injection, they were killed by a second injection of from one-fifth to one-tenth this amount. The moist culture on the basis of 75% water would correspond with 0.062 gm. of the dried organisms. Similarly, dry tubercle bacilli were fatal in dose of 0.02 gm. per 100 gm. of guinea-pig, when given as a first injection. For previously immunized, or sensitized, guinea-pigs, the lethal dose was 0.005 gm. per 100 gm., a fourfold increase in toxicity as regards the treated animal. Here

[5] Ztschr. f. Hyg. u. Infektionskr., 1894, 16, p. 272.

[6] Ztschr. f. Immunitätsf., 1911, 10, p. 467. Centralbl. f. Bakteriol., R., 1911, 50 (Beiheft), p. 58; that is, Bericht üb. d. 5. Tagung d. Fr. Ver. f. Mikrobiol. in Dresden, 1911.

again, the effects were those of anaphylaxis, and were explained as due to the liberation of endotoxin.

Seitz[7] tested the toxicity of 17 different organisms by injecting guinea-pigs with from 5 to 100 mg. of the live cultures. Occasionally he obtained acute fatal shocks, tho usually death was delayed for from 1 to 25 hours. The dysentery bacillus, for example, in dose of 20 mg. killed a 200-gm. guinea-pig in 2 minutes. He concluded that the intravenous injection of saprophytic, as well as of virulent, bacteria caused anaphylactic poisoning, and that death was not due to embolism.

Müller[8] made similar observations with boiled cultures of 4 organisms. He noted that distinct anaphylactic effects, at times acute deaths, were produced in normal animals injected with such material, and that the sensitized animals on reinjection were more liable to acutely fatal shock. The lethal dose for the former, in which acute death resulted, ranged from 7 to 23 mg. of the dried organisms, presumably for guinea-pigs of 200 gm.

These observations are sufficient to show that the dead and the living cultures of various organisms produce essentially the same poisoning, and that the dosage, with the exception of cholera vibrio, is about the same. It has been shown in Table 62 that agar can produce a similar typical shock in dose of 0.94 mg. per 100 gm. of guinea-pig, and that many such deaths can be obtained with from 1 to 2 to 3 mg. It is a rather remarkable fact that a substance apparently as inert as agar should be almost as toxic as the cholera vibrio, and possibly 20 times as toxic as the tubercle bacillus, and 62 times as toxic as Vibrio metschnikovi. The effects produced by the agar are the same, and yet one cannot speak of an agar endotoxin. The same holds true for the intoxications caused by kaolin, and by silicic acid, already mentioned in connection with Table 62.

This fact goes to show that the so-called toxicity of the cholera vibrio, as well as that of the tubercle, typhoid, and dysentery bacilli, pneumococcus, trypanosomes, etc., is of the same order as that of agar. The substance of the invading organism is not a poison, neither is it broken up into a poison by enzymatic action, but the whole cell or its fragments, like agar, kaolin, peptone, etc., induce a change in the plasma which results in the production of anaphylatoxin. The more perfect the comminution of such matter, the more toxic does it seem to be.

[7] Ztschr. f. Immunitätsf., 1911, 11, p. 588.
[8] Ibid., 1912, 14, p. 426.

In other words, much of what in the past has been designated as endotoxin becomes a fiction and thereby loses its specificity. The pathogenic or nonpathogenic organism, dead or alive, as well as noncellular substances (organic and inorganic), acting on serum in the test tube, or on the plasma in the living body, create a disturbance which finds its expression in anaphylatoxic poisoning. One general principle underlies a host of so-called intoxications.

SUMMARY

Agar injected into guinea-pigs may produce a typical anaphylactic shock and death with characteristic autopsy findings.

With agar in the proper physical state this result can be produced by as little as 9.4 mg. of agar per kilo of body weight. This is less than the hitherto observed lethal doses of kaolin or silica, and less than those of most bacteria.

Agar sol, at 50 C., in dose of 10 c.c. (50 mg. of agar) usually is without effect, but it may produce typical shock and death.

Coarse suspensions of gel in salt solution yield atypical shock and findings.

Sol-gel mixtures with distilled water tend to yield an atypical shock; made with salt solution, they produce a typical anaphylactic shock, which is preceded by a definite period of incubation.

The rabbit appears to be somewhat less susceptible than the guinea-pig; 16.6 mg. per kilo caused death.

The rat is still less reactive to agar; it may tolerate from 30 to 50 mg. per kilo, but fatal shocks, preceded by a latent period, were obtained with 27, 32, and 80 mg. per kilo. This insusceptibility of the rat and of the rabbit parallels their behavior to injections of anaphylatoxin (Part V).

The in-vivo production of anaphylatoxin in agar-shocked rats and guinea-pigs (and possibly rabbits) is demonstrable by blood transfusion, and proof is thus given that the shock effects and death are due to this poison.

With a transfer time of 1 minute, the transfusion of 4 c.c. of normal rat blood resulted in typical fatal anaphylactic shock, due to the anaphylatoxin formed as the result of the precoagulation disturbance.

The transfusion method has shown that the blood of normal untreated rabbits may be toxic in dose of 2 c.c., and that the serum from such animals may be fatal in dose of 1 c.c. The apparently healthy rabbit may carry 50 guinea-pig lethal doses per kilo.

This inherent or acquired toxicity is to be correlated with that developed by immunizing injections, also with the precoagulation toxicity, and with that of normal serum, as well as with that induced in normal serum by alien substances. It will eventually be shown to be identical with that produced in specific anaphylactic shock.

A comparison of the toxicity of agar with the values given by Pfeiffer for the cholera endotoxin shows that the 'inert' agar is almost as active. Agar is many times more toxic than the ordinary pathogenic bacteria. The similarity in effects justifies the conclusion that much of the so-called 'endotoxin' of the various pathogenic organisms is of the same order as the toxicity of agar. In short, the common conception of endotoxin is fundamentally wrong.

The disturbance in the plasma caused by the introduction of alien substances results in the formation of anaphylatoxin the poisonous effects of which have been erroneously attributed to a liberation of the so-called endotoxin.